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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 044 683 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
18.10.2000 Bulletin 2000/42

(51) Int Cl.[7]: A61K 9/50, A61K 9/16

(21) Application number: 99107570.6

(22) Date of filing: 15.04.1999

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

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(54) One-step dispersion method for the microencapsulation of water soluble substances

(57) The present invention relates to microcapsules and methods of preparing microparticles with an extremely high encapsulation rate. In a O/W system, by skipping the step of a stable primary emulsion comprising organic solvent droplets, and by carefully setting the physico chemical parameters, microparticles produced by the method of the present invention show surprisingly good agent retention qualities.

Printed by Jouve, 75001 PARIS (FR)

Description

Background of the invention

[0001] Many different methods of preparation of microspheres are described in the literature (Herrmann et al., European Journal of Pharmaceutics and Biopharmaceutics 45 (1998) 75-82). The methods presently used for the preparation of microspheres from hydrophobic polymers are organic phase separation and solvent removal techniques.

[0002] The solvent removal techniques can be divided into solvent evaporation, solvent extraction, spray drying and supercritical fluid technology. In solvent evaporation or solvent extraction techniques, a drug containing organic polymer solution is emulsified into an aqueous or another organic solution. The drug is dissolved, dispersed or emulsified in the inner organic polymer solution.

[0003] These solvent removal techniques for production of microspheres by evaporation or extraction necessitate the step of preparing a stable emulsion of organic droplets before solvent removal. The size and characteristics of the final microspheres depend on this step during which a stable emulsion in the presence of the solvent is a prerequisite. The proportions of organic solvent and aqueous phase in the solvent removal methods are carefully maintained so as to control the solvent migration in the aqueous phase. Below a certain ratio organic solvent/ aqueous phase, the formation of droplets is not possible any more (see H. Sah, "Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres," Journal of controlled release, 47 (3) 1997, 233-245). In some methods, solvent is even added to the aqueous phase in order to saturate it and to prevent the solvent migration during the formation of the primary emulsion.

[0004] Several related patents and published applications describe various aspects of these processes.

[0005] EP 0 052 105 B2 (Syntex) describes a microcapsule prepared by the phase separation technique using a coacervation agent such as mineral oils and vegetable oils.

[0006] EP 0 145 240 B1 (Takeda) discloses a method for encapsulating a water soluble compound by thickening the inner phase of a W/O emulsion, building a W/O/W and subjecting the emulsion to an in water drying process. This method brings different drawbacks such as: the necessity of using a thickening agent to retain the drug, and the multi step procedure including two emulsification steps and the in water drying step.

[0007] EP 0 190 833 B1 (Takeda) describes a method for encapsulating a water soluble drug in microcapsules by increasing the viscosity of a primary W/O emulsion to 150-5'000 c p (by the procedure of increasing the polymer concentration in the organic phase or by adjusting the temperatures) prior to formation of a second W/O/W emulsion which is then subjected to in water drying. The drawbacks of this procedure are the complexity of the necessary steps, including formation of two emulsions (W/O and W/O/W) one after the other, and the in water drying.

[0008] US 5,407,609 (Tice/SRI) describes a microencapsulation process for highly water soluble agents. This process involves the distinct steps of forming a primary O/W emulsion, the external aqueous phase being preferably saturated with polymer solvent. This O/W emulsion is then poured to a large volume of extraction medium in order to extract immediately the solvent. The drawback of this method is that the O/W emulsion is formed in the presence of the organic solvent in a small volume. The solvent is subsequently removed by extraction in a large aqueous volume. The polymeric droplets are prevented to harden in the primary emulsion, allowing the migration of the drug into the external phase.

[0009] WO 95/11008 (Genentech) describes a method for the encapsulation of adjuvants into microspheres. The process comprises the three distinct steps of preparing a primary W/O emulsion, followed by the production of a W/O/W and finally the hardening of the microspheres by extraction of the solvent. As already mentioned above, the drawback of such a method is the complication due to a multistep procedure separating droplet production from solvent elimination.

[0010] EP 0 779 072 A1 (Takeda) describes an in-water drying method used for the removal of solvent after production of a W/O/W or a O/W emulsion. It is mentioned that the O/W method is preferable for active substances insoluble or sparingly soluble in water.

[0011] It is an object of the present invention to present a new method for the production of microparticles comprising water soluble agents.

[0012] It is also an object of the present invention to create new microparticles comprising water soluble active substances.

[0013] It is still further an object of the present invention to produce a new method for the production of microparticles of high encapulating efficiency comprising water soluble active substances.

[0014] It is further an object of the present invention to present a method which allows for a reduction of time of exposure of water soluble active substances to solvent in the production of microparticles.

[0015] It is further an object of the present invention to avoid the formation of specific emulsions, and the problems they have caused as described in the prior art in the production of microparticles comprising water soluble active substances.

Summary of the invention

[0016] The present invention relates to microcapsules and a method of preparing them with an extremely high encapsulation rate thanks to the optimal reduction of dif-

fusion for the compound or agent to be encapsulated.

[0017] The methods available up to now for encapsulating certain compounds and agents, and particularly, water soluble compounds and agents, were not efficient enough for encapsulating water soluble active substances due to the high affinity that water soluble active substances have with the aqueous phase. The present invention has found a solution to this problem by reducing the time required for encapsulating water soluble actives substances, and therefore avoiding the problem of formation of the primary emulsion and solvent removal steps which were far too long and allowed the migration of the water soluble agents into the external aqueous phase.

[0018] In the method of the present invention, it has been surprisingly discovered that it is possible to obtain microparticles with an extremely high encapsulation efficiency of water soluble active substances using a new one step O/W homogenization process.

[0019] The basic method of the present invention can be simply characterized as providing:

- using a volume of aqueous phase in an O/W system capable of dissolving at once the total amount of organic solvent used;
- working with water soluble compounds or agents dispersed in an organic phase, the agent preferably present in solid state in the inner phase during the entrapment procedure (slowing down the solubilisation into the aqueous phase);
- carefully setting the physico chemical parameters during the homogenization of the organic inner phase into the aqueous phase.

[0020] By skipping the step of a stable primary emulsion comprising organic solvent droplets, and by carefully setting the physico chemical parameters such as surfactant capacity, viscosity, temperature, ionic strength, pH and buffering potential, the specific embodiments of the present invention show surprisingly good agent retention qualities.

[0021] Furthermore, since the polymer precipitates because of the instantaneous lack of solvent, imprisoning the active principle, no emulsion stage is seen. The particles can thus be directly harvested after their formation.

[0022] Because the particle formation and the solvent removal are done together in one single step in this method, the water soluble active substance is at once kept inside the particles which have an impermeable wall. Thereby any diffusion external to the particle is at a low level, and the encapsulation rate is very high. This one single process also avoids the steps of extraction and of solvent evaporation.

Detailed description of the invention

[0023] By adjusting various production parameters, various embodiments of the present invention can be obtained. In one of the preferred embodiments, by carefully adjusting the production parameters, the precipitating polymer can be surprisingly well formed into homogeneously dispersed particles. The amount of organic solvent is thereby kept as low as possible to get a viscous solution of the polymer and to minimize the necessary volume of the aqueous phase.

[0024] In preferred embodiments, the volume of the aqueous phase is chosen to be capable of dissolving at least the complete amount of organic solvent. A surfactant is added to the aqueous phase in order to keep the precipitating polymer in fine independent particles. An ideal surfactant gives a viscosity to the aqueous phase that approaches the viscosity of the organic phase. An electolyte may also be optionally added to the aqueous solution to create repulsion between the particles and preventing aggregation. The aqueous solution can also be buffered to obtain good pH conditions for the drug concerning stability and release.

[0025] In further preferred embodiments, the encapsulation efficiency is increased when using cold solutions, by optimizing the solubility of the solvent in water, by reducing the aqueous solubility of the drug, and by slowing down its diffusion. In other words, in embodiments using solutions of lower temperature, the present invention achieves the effect of further reducing the already small amount of diffusion of internal particle substances to the exterior. In these and other preferred embodiments, after homogenization, the dispersion is directly filtered. The particles are then harvested and lyophilized.

Detailed Description of the Preferred Methods

[0026] The aim of the invention is to provide a microparticle and a method for the preparation of such microparticle having a high encapsulating efficiency of a water soluble substance in a biodegradable polymer obtained by a one step dispersion process of two non miscible phases, an organic phase (solvent) containing the water soluble substance and the polymer into an aqueous phase containing a surfactant and optionally salts. The particle formation and their hardening is performed in one single step. The harvesting of the particles is done directly after their formation. The amount of the organic phase (solvent) is kept as low as possible in order to be dissolved at once into the aqueous phase. Regarding to this method the encapsulation efficiency is greater than 50 % and preferably greater than 80 %.

[0027] A physiologically active peptide, or protein or any other active principle is dispersed in an organic solvent which is not miscible with water such as ethyl acetate, methylene chloride, diethylcarbonate, chloroform or the like. The size of the active principle solid particles can be adjusted if necessary by homogenization.

[0028] The organic solution containing the active principle is used to dissolve the biodegradable polymer

comprising poly(lactides), poly(glycolides), copolymers thereof or other biodegradable polymers such as other aliphatic polymers, polycitric acid, polymalic acid, polysuccinates, polyfumarates, poly-hydroxybutyrates, polycaprolactones, polycarbonates, polyesteramides, polyanhydrides, poly (amino acids), polyorthoesters, polycyanoacrylates, polyetheresters, poly(dioxanone)s, copolymers of polyethylene glycol (PEG), polyorthoesters, biodegradables polyurethanes, polyphosphazenes.

[0029] Other biocompatible polymers are polyacrylic acid, polymethacrylic acid, acrylic acid-methacrylic acid copolymers, dextran stearate, ethylcellulose, acetyl-cellulose, nitrocellulose, etc. These polymers may be homopolymers or copolymers of two or more monomers, or mixtures of the polymers.

[0030] The peptide and the polymer can also be prepared in separate organic solutions. The polymer is dissolved in an organic solvent which is not miscible with water. Preferred solvents include ethyl acetate or methylene chloride; more preferred is when the solvent used to dissolve the polymer is the same solvent as that use for suspending the peptide.

[0031] The organic solutions are poured together to form a homogenous organic suspension (organic phase) before addition to the aqueous phase.

[0032] The organic solvent(s) used may also be any organic solvent hardly miscible with water, i.e. having a low solubility in water.

[0033] Examples of such preferred solvents include diethyl carbonate, ethyl acetate, methylene chloride other halogenated hydrocarbons (e.g. dichloromethane, chloroform, chloroethane, dichloroethane, trichloroethane, carbon tetrachloride, fatty acid esters (e.g. ethyl acetate, butyl acetate, ethers (e.g. ethyl ether, isopropyl ether and aromatic hydrocarbons (e.g. benzene, toluene, xylene) or the like. Preferably, the preferred solvents can be alone or used in mixtures of two or more solvents. More preferably, the solvent is ethyl acetate. If the polymer is not or is only slightly soluble in ethyl acetate, co-solvents, comprised among the family of benzyl alcohol, DMSO, DMF, ethyl alcohol, methyl alcohol, acetonitrile and the like may optionally be used in that instance.

[0034] The peptide can be dissolved first in a (any commercially available) solvent like dimethylsulfoxide in order to allow a sterile filtration. Once this solution is added to the polymer solution, the peptide precipitates while the dimethylsulfoxide mixes with the organic solvent.

[0035] Preferably, the amount of solvent used to dissolve the biodegradable polymer is kept to a minimum in order to be soluble as quickly as possible (most preferably at once) in the aqueous phase. (If the amount of solvent is high, the amount of aqueous phase has to be too large on a practical point of view).

[0036] The concentration of polymer in the organic phase is adjusted to 5-90% (by weight), preferably between about 10 and 50%, depending on the polymer and solvent used.

[0037] In the case that the concentration of polymer in the organic solvent is high, the viscosity of this phase, depending on the polymer used, may be increased.

[0038] The viscosity of the polymer solution may be comprised between 1000 and 40'000 centipoise (cp) (Brookfield viscosity), more preferably between 2,000 and 30,000 c p, even more preferable between 3'000 and 20'000 c p.

[0039] Using ethyl acetate for dissolving the polymer, the solubility of the solvent in the aqueous phase is increased by lowering the temperature of both, the organic and the aqueous phases, accelerating the solvent migration and therefore also the encapsulation rate.

[0040] In methods of the present invention, the temperature of the organic phase ranges between about -10°C and 30°C, and preferably between about 0°C and 10°C even more preferably between about 2°C and 5°C. The temperature of the polymeric organic phase and the temperature of the aqueous phase are the same or different and are adjusted in order to increase the solubility of the solvent in the aqueous phase.

[0041] The obtained organic solution for use as the inner polymer and drug containing phase is added to a aqueous outer phase under a homogenisation procedure to give microparticles.

[0042] For the homogenisation procedure, a method of creating dispersion is used. This dispersion can be realized for example with any apparatus capable of shaking, mixing, stirring, homogenizing or ultrasonicating.

[0043] Different agents influencing the physico-chemical characteristics of the (resultant) solution may be added : surfactants, such as for example an anionic surfactant (e.g. sodium oleate, sodium stearate, sodium lauryl sulfate), a nonionic surfactant [(e.g. polyoxyethylenesorbitant fatty acid ester (Tween 80, Tween 60, products of Atlas Powder Co, U.S.A.), a polyoxyethylene castor oil derivative (HCO-60, HCO-50, products of Nikko Chemicals, Japan)], polyvinyl pyrrolidone, polyvinyl alcohol, carboxymethylcellulose, lecithin or gelatin.

[0044] In specific embodiments of the present invention, a surfactant comprised among the family of anionic, nonionic agents or other agents capable of reducing the surface tension of the polymeric dispersion can be added. Suitably, therefore, are nonionic surfactants such as Tween (for example Tween 80), anionic surfactants, nonionic surfactant like polyvinyl alcohol or others. These surfactants can, in general, be used alone or in combination with other suitable surfactants. The concentration of the surfactant is selected in order to disperse and stabilize the polymer particles, and possibly also to give a viscosity approaching the viscosity of the organic phase.

[0045] The preferred concentration of the surfactant in the aqueous phase ranges therefore between about 0.01-50% (by weight), preferably between about 5 and 30%. The viscosity depending on the surfactant used

and on its concentration ranges between about 1'000-8'000cp (Brookfield viscosity), preferably about 3'000-5'000 cp.

[0046] Optionally salts comprised among the family of sodium chloride, Potassium chloride, carbonates, phosphates and the like can be added to the aqueous phase to adjust ionic strength and to create a Zeta potential between the polymer particles, leading to particle repulsion. Additional buffering agents may be added to the aqueous phase to maintain a specific pH. So, the internal aqueous phase may be supplemented with a pH regulator for retaining stability or solubility of a physiologically active substance, such as carbonic acid, acetic acid, oxalic acid, citric acid, phosphoric acid, hydrochloric acid, sodium hydroxide, arginine, lysine or a salt thereof. The pH of the formulations of this invention is generally about 5 to 8, preferably about 6.5 to 7.5. The temperature of the aqueous solution can be adjusted to the temperature of the inner organic phase. The temperature range is from about -10°C to 30°C, more preferably between 0° and 10° C and even more preferably from between 2°C and 5°C

[0047] The microparticles of the present invention can be prepared in any desired size, ranging from 1µm to about 500µm, by varying the parameters such as polymer type and concentration in the organic phase, volumes and temperature of the organic and aqueous phase, surfactant type and concentration, homogenization time and speed. The mean particle size of the microparticles ranges generally from 10 to 200µm, more preferably from 20 to 200µm, even more preferably from 30 to 150µm.

[0048] A number of water soluble active substances can be encapsulated by the method of the present invention. Preferably, the encapsulated soluble substance is a peptide, a polypeptide, a protein and their related salts. The salt of peptide is preferably a pharmacologically acceptable salt. Such salts include salts formed with inorganic acids (e.g. hydrochloric acid, sulfuric acid, nitric acid), organic acids (e.g. carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid) etc. More preferably, the salt of peptide is a salt formed with an organic add (e.g. carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid) with greater preference given to a salt formed with acetic acid. These salts may be mono-through tri-salts. Examples of water soluble active substances which can be encapsulated in the present invention include, but are not limited to, peptides, polypeptides and proteins such as luteinizing hormone releasing hormone (LHRH) or derivatives of LHRH comprising agonists or antagonists, melanocyte stimulating hormone (MSH), thyrotropin releasing hormone (TRH), thyroid stimulating hormone (TRH), follicule stimulating hormone (FSH), human chorionic gonadotropin (HCG), parathyroid hormone (PTH), human placental lactogen, insulin, somatostatin and derivatives, gastrin, prolactin, adrenocorticotropic hormone (ACTH), growth hormones (GH), growth hormone releasing hormone (GHRH), growth hormone releasing peptide (GHRP), calcitonin, oxytocin, angiotensin, vasopressin, enkephalins, endorphin, enkephalin, kyotorphine, interferons, interleukins, tumor necrosis factor (TNF), erythropoetin (EPO), colony stimulating factors (G-CSF, GM-CSF, M-CSF), thrombopoietin (TPO), platelet derived growth factor, fibroblast growth factors (FGF), nerve growth factors (NGF), insulin like growth factors (IGF), amylin peptides, leptin, RGD peptides, bone morphogenic protein (BMP), substance P, serotonin, GABA, tissue plasminogen activator (TPA), superoxide dismutase (SOD), urokinase, kallikrein, glucagon, human serum albumin, gamma globulin, immunomodulators (EGF, LPS), blood coagulating factor, lysozyme chloride, polymyxin B, colistin, gramicidin, bacitracin and the like.

[0049] A number of other unlimiting example of water soluble compounds or particularly a water soluble form of the following compounds can be encapsulated by the method of the present invention.

[0050] Other unlimiting examples include anticancer drugs such as actinomycin D, bleomycin, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, doxorubicin, estramustine, etoposide, floxuridine, fludarabine, fluorouracil, hexamethylmelamine, hydroxyurea, idarubicin, ifosfamide, asparaginase, lomustine, mechlorethamine, melphalan, mercaptopurine, methotrexate, mithramycin, mitomycin C, mitotane, mitozantrone, pentostatin, procarbazine, streptozocin, teniposide, thioguanine, thiopeta, vinblastine, vincristine and the like; antibiotics such as tetracyclines, penicillins, sulfisoxazole, ampicillin, cephalosporins, erytromycin, clindamycin, isoniazid, amikacin, chloramphenicol, streptomycin, vancomycin and the like.

[0051] Other examples including antivirals such as acyclovir, amantadine, and the like; antipyretics, analgesics and antiinflammatory agents include acetaminophen, acetylsalicylic acid, methylprodnisolone, ibuprofen diclofenac sodium, indomethacin sodium, flufenamate sodium, pethidine hydrochloride, levorphanol tartrate, morphine hydrochloride, oxymorphone and the like; anesthetics such as lidocaine, xylocaine and the like; antiulcer agents include metoclopramide, ranitidine hydrochloride, cimetidine hydrochloride, histidine hydrochloride, and the like anorexics such as dexedrine, phendimetrazine tartrate, and the like; antitussives such as noscapine hydrochloride, dihydrocodeine phosphate, ephedrine hydrochloride, terbutaline sulfate, isopreterenol hydrochloride, salbutamol sulfate, and the like; antiepileptics such as acetazolamide sodium, ethosuximide, phenytoin sodium, diazepam and the like; antidepressants such as amoxapine, isocarboxamide, phenelzine sulfate, clomipramine, noxiptilin, imipramine, and the likeanticoagulants such as heparin or warfarin, and the like.

[0052] Other unlimiting examples include sedatives

such as chlorpromazine hydrochloride, scopolamine methylbromide, antihistaminics such as diphenhydramine hydrochloride, ketotifen fumarate, chlorpheniramine maleate, methoxyphenamine hydrochloride and the like.'

[0053] Other unlimiting examples include cardiotonics such as etilefrine hydrochloride, aminophylline and the like; antiasthmatics such as terbutaline sulfate, theophylline, ephedrine, and the like; antifungals such as amphotericin B, nystatin, ketoconazole, and the like; antiarrhytmic agents such as propranolol hydrochloride, alprenolol hydrochloride, bufetolol hydrochloride, oxyprenolol hydrochloride and the like; antitubercular agents such as isoniazid, ethambutol, and the like; hypotensive, diuretic agents such as captopril, ecarazine, mecamylamine hydrochloride, clonidine hydrochloride, bunitrolol hydrochloride and the like; hormones such as prednisolone sodium sulfate, betamethasone sodium phosphate, hexestrol phosphate, dexamethasone sodium sulfate and the like; antigens from bacteria, viruses or cancers, antidiabetics such as glipizide, phenformin hydrochloride, buformin hydrochloride, glymidine sodium, methformin, and the like; cardiovascular agents such as propanolol hydrochloride, nitroglycerin, hydralazine hydrochloride, prazosin hydrochloride and the like; diuretics such as spironolactone, furosemide and the like; and enzymes, nucleic acids, plant extracts, anti-malarials, psychotherapeutics, hemostatic agents, etc.

Examples

[0054] The examples that follow are set forth as an aid in understanding the present invention, and provide some examples of the many embodiments that are potentially available for the present invention. They are not intended to limit the scope of the invention.

Example 1.

[0055] 62.5 mg of D-Trp^6-LHRH acetate (Triptorelin acetate) was added to 20g of ethyl acetate. The peptide particles were reduced in size with a small size dispersing apparatus at 20'000 rpm during 5 minutes. This peptide suspension was added to 2 g of poly(D-L-lactide-co-glycolide) (PLGA) with a ratio of lactide to glycolide of 50:50 and a weight average molecular weight of 45'000. The mixture was stirred at room temperature until the polymer was dissolved and then placed still at 4°C. The Brookfield viscosity of this solution was 15'500cp (15.5 Pas).

[0056] This organic phase was poured into 675 g of aqueous phase containing 20% (W/W) of Tween 80 and 7g of sodium chloride and having a temperature of 4°C. The homogenization was performed with a Polytron homogenizer at 4'000 rpm during 3 minutes.

[0057] The microparticles were collected right after the end of the homogenization step by filtration. The microparticles were then vacuum dried at room temperature during 48 hours.

[0058] The entrapment efficiency was 93%, the mean particle size was 52µm, and the residual ethyl acetate was 183ppm (as determined by GC-MS).

Example 2.

[0059] 1250 mg of D-Trp^6-LHRH acetate (Triptorelin acetate) was added to 200g of ethyl acetate. The peptide particles were reduced in size with a small size dispersing apparatus at 20'000 rpm during 5 minutes.

[0060] 40 g of poly(D-L-lactide-co-glycolide) (PLGA) with a ratio of lactide to glycolide of 50 : 50 and a weight average molecular weight of 45'000 were dissolved in 200g of ethyl acetate at room temperature.

[0061] Both organic phases were poured together and stirred briefly on a magnetic stirrer. The suspension was then let to stand at 4°C until use. This organic phase was poured into 7kg of aqueous phase containing 20% (W/W) of Tween 80 in 67mM phosphate buffer pH 7.4 and 70 g of sodium chloride and having a temperature of 4°C. The homogenization was performed at 2'000 rpm during 5 minutes.

[0062] The microparticles were collected right after the end of the homogenization step by filtration. The microparticles were then vacuum dried at room temperature during 48 hours.

[0063] The entrapment efficiency was 76% and the mean particle size was 150µm.

Example 3.

[0064] 125 mg of bovine serum albumin was added to 20g of ethyl acetate. The solid protein particles were reduced in size with a small size dispersing apparatus at 20'000 rpm during 5 minutes. This protein suspension was added to 2 g of poly(D-L-lactide-co-glycolide) (PLGA) with a ratio of lactide to glycolide of 50 :50 and a weight average molecular weight of 45'000. 20g of additional ethyl acetate were added. The mixture was stirred at room temperature until the polymer was dissolved and then placed still at 4°C.

[0065] This organic phase was poured into 675 g of aqueous phase containing 20% (W/W) of Tween 80 in 67mM phosphate buffer pH 7.4 and 7g of sodium chloride and having a temperature of 7°C. The homogenization was performed with a Polytron homogenizer at 4'000 rpm during 3 minutes.

[0066] The microparticles were collected right after the end of the homogenization step by filtration.

[0067] The microparticles were then vacuum dried at room temperature during 48 hours. The entrapment efficiency was 76% and the mean particle size was 74µm.

Example 4.

[0068] 125 mg of D-Trp^6-LHRH acetate (Triptorelin

acetate) was added to 5g of ethyl acetate. The peptide particles were reduced in size with a small size dispersing apparatus at 20'000 rpm during 5 minutes.

[0069] 4 g of poly(D-L-lactide) polymer were added to this peptide suspension. The mixture was stirred at room temperature until the polymer was dissolved and then placed still at 8°C. This organic phase was poured into 675 g of aqueous phase containing 20% (W/W) of Tween 80 in 67mM phosphate buffer pH 7.4 and 7 g of sodium chloride and having a temperature of 5°C. The homogenization was performed with a homogenizer at 4'000 rpm during 3 minutes.

[0070] The microparticles were collected right after the end of the homogenization step by filtration.

[0071] The microparticles were then vacuum dried at room temperature during 48 hours. The entrapment efficiency was 57% and the mean particle size was 30µm.

Claims

1. A microparticle with a high encapsulation efficiency of a water soluble substance in a biodegradable polymer obtained by a one step dispersion process of an organic phase containing said soluble substance and said polymer and an aqueous phase containing a surfactant and optionally salts, characterized in that the amount of the organic phase (solvent) is kept as low as possible in order to be dissolved at once into the aqueous phase.

2. A method for producing microparticles with a high encapsulation efficiency of a water soluble substance in a biodegradable polymer obtained by a one step dispersion process of an organic phase containing said soluble substance and said polymer and an aqueous phase containing a surfactant and optionally salts, characterized in that the amount of the organic phase (solvent) is kept as low as possible in order to be dissolved at once into the aqueous phase.

3. The method of claim 2, wherein the encapuslation efficiency is greater than 50%.

4. The method of claim 2, wherein the solvent presents a low solubility in water and is comprised among the family of ethyl acetate, methylene chloride, diethyl carbonate, chloroform or the like.

5. The method of claim 2 wherein the encapsulated soluble substance is a peptide, a polypeptide, a protein and their related salts or a compound af any other structure among analgesics, anticancer drugs, antibiotics, antivirals, antipyretics, analgesics, antiinflammatory agents, anesthetics, antiulcer agents, anorexics, antitussives, antiepileptics, antidepressants, anticoagulants, sedatives, cardiotonics, antiasthmatics, antifungals, antiarrhytmic agents. antitubercular agents, hypotensive, diuretic agents. hormones, antigens from bacteria, viruses or cancers, antidiabetics, cardiovascular agents, diuretics, enzymes, nucleic acids, plant extracts, antimalarials, psychotherapeutics, hemostatic agents or others.

6. The method of claim 2, wherein the solvent may optionally be mixed with a co-solvent comprised among the family of DMSO, DMF, ethyl alcohol, methyl alcohol, acetonitrile and the like.

7. The method of claim 2, wherein the surfactant is comprised among the family of anionic, nonionic agents or other agents capable of reducing the surface tension of the polymeric dispersion.

8. The method of claim 2, wherein the salts are comprised among the family of sodium chloride, Potassium chloride, carbonates, phosphates and the like.

9. The method of claim 2, wherein the temperature of the polymeric organic phase and the temperature of the aqueous phase are the same or different and are adjusted in order to increase the solubility of the solvent in the aqueous phase.

10. The method of claim 2, wherein the biodegradable polymer is a poly(lactide), poly(glycolide), copolymers thereof or any other suitable biocompatible polymer.

11. The method of claim 2, wherein the concentration of the polymer in the organic phase is comprised between 5 to 90 % (by weight).

12. The method of claim 2, wherein the concentration of the polymer in the organic phase creates a viscosity comprised between 1'000 and 40'000 centipoise.

13. The method of claim 2, wherein the concentration of the surfactant in the aqueous phase is comprised between 0.01 - 50% (by weight) and creates a viscosity comprised between 1'000 and 8'000 centipoise.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 99 10 7570

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	GB 2 257 909 A (DEBIO RECH PHARMA SA) 27 January 1993 (1993-01-27) * page 5, line 24 - page 5, line 28; examples 1-8 *	1-13	A61K9/50 A61K9/16
X	GB 2 234 896 A (SANDOZ LTD) 20 February 1991 (1991-02-20) * examples 1-3,11 *	1-13	
X	EP 0 585 151 A (RHONE MERIEUX) 2 March 1994 (1994-03-02) * examples 1-3 *	1-13	
X	WO 97 41837 A (JANSSEN PHARMACEUTICA NV ;ALKERMES INC (US)) 13 November 1997 (1997-11-13) * example 1 *	1-13	
X	GB 1 297 476 A (FARBENFABRIKEN BAYER AG) 22 November 1972 (1972-11-22) * example 1 *	1-13	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
	-/--		A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	2 December 1999	Pilling, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 99 10 7570

Claim(s) searched incompletely:
1-13

Reason for the limitation of the search:

It is considered that a lack of clarity of the present claims arises to such an extent as to render a meaningful search of the full scope of the present claims impossible (see the Guidelines, B-III, 3.12 and B-VIII, 6).

In this regard, Claim 1 is directed towards a "microparticle with a high encapsulation efficiency..//.. obtained by a one step dispersion process..Etc". These latter technical features are considered to be process features and do not clearly characterize the product of Claim 1. In this regard the use of process features to characterize a product is unclear (see the Guidelines C-III, 4.7b).

Furthermore, with reference to Claim 2; the reference to "high encapsulation efficiency" and the statement that the "amount of the organic phase (solvent) is kept as low as possible in order to be dissolved at once into the aqueous phase" are also considered to be vague and equivocal (see the Guidelines C-III, 4.5).

It is further noted that the organic solvents referred to in the present application (see, for example the solvents of present Claim 4) are immiscible with water (see also present page 8 lines 21 to 26). Hence, the reference to said organic solvent being "dissolved at once into the aqueous phase" in Claim 1 and 2 is in contradiction with the the use of said immiscible solvents.

Moreover, Claims 3 to 10 either fail to define more clearly the invention of Claim 2 or relate to features which are vague and unclear. Hence, it is not possible to carry out a meaningful search on the basis of the invention as defined in any of Claims 1 to 10. In this regard, it is noted, for example that Claim 5 defines, for example, the use of any soluble substance and Claim 7 defines any surfactant.

Similarly, the features of Claims 11 to 13 relate to unsearchable parameters.

Consequently, the search has been carried out for those features of the claims i) which relate to potentially patentable subject matter and ii) which are supported by the present examples and iii) for which a which a search is feasible, i.e:

a method for producing microparticles comprising a water-soluble substance in a biodegradable poly(lactide) and/or poly(glycolide) polymer/copolymer wherein formation of said particles is achieved by dissolving or dispersing an organic phase containing said soluble substance and said polymer/copolymer in an aqueous phase containing a surfactant.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 99 10 7570

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	US 5 238 714 A (WALLACE MICHAEL ET AL) 24 August 1993 (1993-08-24) * column 2, line 58 - column 2, line 63; example 1 *	1-13	
X	US 3 691 090 A (KITAJIMA MASAO ET AL) 12 September 1972 (1972-09-12) * examples 1-9 *	1-13	
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